

Please type a plus (+) inside this box -

PTO/SB/29 (12/97)

Approved for use through 09/30/00. OMB 0651-0032

Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

# UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	9882-015-999	Total Pages	139
First Named Inventor or Application Identifier			
Gonzalez			
Express Mail Label No.		EL 501 637 999 US	

## APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

- |   |   |   |
|---|---|---|
| 1. <input checked="" type="checkbox"/> Fee Transmittal Form<br><i>Submit an original, and a duplicate for fee processing</i>  | 6. <input type="checkbox"/> Microfiche Computer Program (Appendix)  |   |
| 2. <input checked="" type="checkbox"/> Specification<br><i>(preferred arrangement set forth below)</i>  | 7. <input type="checkbox"/> Nucleotide and/or Amino Acid Sequence Submission<br><i>(if applicable, all necessary)</i>                                 |   |
| -Descriptive title of the Invention   | a. <input type="checkbox"/> Computer Readable Copy  |   |
| -Cross Reference to Related Applications  | b. <input type="checkbox"/> Paper Copy (identical to computer copy)   |   |
| -Statement Regarding Fed sponsored R&D  | c. <input type="checkbox"/> Statement verifying identity of above copies  |   |
| -Reference to Microfiche Appendix   |   |   |
| -Background of the Invention  |   |   |
| -Brief Summary of the Invention   |   |   |
| -Brief Description of the Drawings <i>(if filed)</i>  |   |   |
| -Detailed Description of the Invention (including drawings, <i>if filed</i> )   |   |   |
| -Claim(s)   |   |   |
| -Abstract of the Disclosure   |   |   |
| 3. <input checked="" type="checkbox"/> Drawing(s) <i>(35 USC 113)</i>   | [Total Sheets 19]   | 8. <input type="checkbox"/> Assignment Papers (cover sheet & document(s))   |
| 4. <input checked="" type="checkbox"/> Oath or Declaration (Unexecuted)   | [Total Sheets 2]  | 9. <input type="checkbox"/> 37 CFR 3.73(b) Statement <input type="checkbox"/> Power of Attorney<br><i>(when there is an assignee)</i> |
| a. <input type="checkbox"/> Newly executed (original or copy)   |   |   |
| b. <input type="checkbox"/> Copy from a prior application (37 CFR 1.63(d))<br><i>(for continuation/divisional with Box 17 completed)</i>  |   |   |
| i. <input type="checkbox"/> <b><u>DELETION OF INVENTORS(S)</u></b><br>Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33 (b).  | 10. <input type="checkbox"/> English Translation Document <i>(if applicable)</i>  |   |
| 5. <input type="checkbox"/> Incorporation By Reference <i>(useable if Box 4b is checked)</i><br>The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein. | 11. <input type="checkbox"/> Information Disclosure Statement (IDS)/PTO-1449 <input type="checkbox"/> Copies of IDS Citations                         |   |
| 17. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information:<br><input type="checkbox"/> Continuation <input type="checkbox"/> Divisional <input type="checkbox"/> Continuation-in-part (CIP)    of prior application No: filed .   | 12. <input type="checkbox"/> Preliminary Amendment  |   |
|   | 13. <input checked="" type="checkbox"/> Return Receipt Postcard (MPEP 503)<br><i>(Should be specifically itemized)</i>                                |   |
|   | 14. <input type="checkbox"/> Small Entity <input type="checkbox"/> Statement filed in prior application, Statement(s) Status still proper and desired |   |
|   | 15. <input type="checkbox"/> Certified Copy of Priority Document(s)<br><i>(if foreign priority is claimed)</i>  |   |
|   | 16. <input type="checkbox"/> Other:   |   |

## ACCOMPANYING APPLICATION PARTS

8.  Assignment Papers (cover sheet & document(s))
9.  37 CFR 3.73(b) Statement  Power of Attorney  
*(when there is an assignee)*
10.  English Translation Document *(if applicable)*
11.  Information Disclosure Statement (IDS)/PTO-1449  Copies of IDS Citations
12.  Preliminary Amendment
13.  Return Receipt Postcard (MPEP 503)  
*(Should be specifically itemized)*
14.  Small Entity  Statement filed in prior application, Statement(s) Status still proper and desired
15.  Certified Copy of Priority Document(s)  
*(if foreign priority is claimed)*
16.  Other:

## 17. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information:

Continuation     Divisional     Continuation-in-part (CIP)    of prior application No: filed .

## 18. CORRESPONDENCE ADDRESS

<input checked="" type="checkbox"/> Customer Number or Bar Code Label	20583  (Insert Customer No. or Attach bar code label here)	or <input type="checkbox"/> Correspondence address below			
NAME	Pennie & Edmonds LLP				
ADDRESS	1155 Avenue of the Americas 17 <sup>th</sup> Floor				
CITY	New York	STATE	NY	ZIP CODE	10036-2711
COUNTRY	USA	TELEPHONE	(212) 790-9090		FAX (212) 869-8864

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231.



03/13/01

03-14-01

Express Mail No.: EL 501 637 999 US

## PENNIE &amp; EDMONDS LLP

COUNSELLORS AT LAW  
 1155 Avenue of the Americas  
 New York, N.Y. 10036-2711  
 (212) 790-9090

A

ATTORNEY DOCKET NO. 9882-015-999

Date: March 13, 2001

JC973 U.S. PTO  
 09/805353  
 03/13/01

Assistant Commissioner for Patents  
 Box PATENT APPLICATION  
 Washington, D.C. 20231

Sir:

The following utility patent application is enclosed for filing:

Applicant(s): Gonzalez et al.

Executed on: March 13, 2001

Title of Invention: ENGINEERED PROTEIN BINDING DOMAINS AND METHODS AND SYSTEMS FOR THEIR DESIGN AND USE

## PATENT APPLICATION FEE VALUE

TYPE	NO. FILED	LESS	EXTRA	EXTRA RATE	Fee
Total Claims	133	-20	113	\$18.00 each	\$ 2,034.00
Independent	27	-3	26	\$80.00 each	\$ 2,080.00
				Minimum Fee	\$ 710.00
				Multiple Dependency Fee If Applicable (\$270.00)	\$ 0.00
				Total	\$ 4,824.00
				50% Reduction for Independent Inventor, Nonprofit Organization or Small Business Concern (a verified statement as to the applicant's status is attached)	- \$ 0.00
				Total Filing Fee	\$ 4,824.00

- DO NOT PUBLISH.** I hereby certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral agreement, that requires publication at eighteen months after filing. I hereby request that the attached application not be published under 35 U.S.C. 122(b).
- Priority of application no. filed on is claimed under 35 U.S.C. § 119.
- The certified copy of the priority application has been filed in application no. filed
- Amend the specification by inserting before the first line the following sentence: This is a continuation-in-part of application no. filed .

Please charge the required fee to Pennie & Edmonds LLP Deposit Account No. 16-1150. A copy of this sheet is enclosed.

Respectfully submitted,

Adriane M. Antler  
 PENNIE & EDMONDS LLP  
 Enclosure

Respectfully submitted,

By  
 Dwight H. Renfrew, Jr.  
 PENNIE & EDMONDS LLP

32,605

(Reg. No.)

38,594

(Reg. No.)

**ENGINEERED PROTEIN BINDING DOMAINS  
AND METHODS AND SYSTEMS  
FOR THEIR DESIGN AND USE**

5

**TABLE OF CONTENTS**

1.	<u>FIELD OF THE INVENTION</u>	1
10	2. <u>BACKGROUND OF THE INVENTION</u>	1
15	3. <u>SUMMARY OF THE INVENTION</u>	3
20	4. <u>BRIEF DESCRIPTION OF THE FIGURES</u>	13
25	5. <u>DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT</u>	15
30	5.1. <u>DOMAIN ENGINEERING/REDESIGN METHODS</u>	15
35	5.1.1. <u>PREFERRED PRECURSOR DOMAINS</u>	15
	5.1.2. <u>GENERAL ENGINEERING/REDESIGN METHODS</u>	18
	5.1.3. <u>PREFERRED TARGETS</u>	24
	5.1.4. <u>PREFERRED PRECURSORS</u>	27
	5.1.5. <u>SELECT CANDIDATE DOMAINS</u>	31
	5.1.6. <u>SCREENING CANDIDATE DOMAINS</u>	35
	5.2. <u>SCREENING METHODS</u>	44
	5.2.1 <u>RECONSTITUTION METHODS</u>	45
	5.2.1.1 <u>PROTEIN-PAIR RECONSTITUTION METHODS</u>	45
	5.2.1.2 <u>LIBRARY-PAIR RECONSTITUTION METHODS</u>	47
	5.2.2 <u>DIRECT OBSERVATION OF BINDING</u>	51
	5.2.2.1 <u>AFFINITY CHROMATOGRAPHY</u>	52
	5.2.2.2 <u>BIOSENSOR ANALYSIS</u>	54
	5.2.2.3 <u>PHYSICAL METHODS</u>	57
	5.2.2.3 <u>PHAGE DISPLAY</u>	58
	5.2.2.4 <u>RNA-PROTEIN FUSIONS</u>	59
	5.3. <u>DOMAIN SYNTHESIS</u>	61
	5.3.1 <u>PREPARATION/MUTAGENESIS OF ENCODING DNA</u>	61
	5.3.2 <u>PROTEIN SYNTHESIS</u>	67

Page

5.4.	<u>SYSTEMS OF THE INVENTION</u>	68
5.5.	<u>USES OF ENGINEERED BINDING PROTEINS</u>	70
5	5.5.1 <u>SINGLE DOMAINS</u>	70
	5.5.1.1 <u>PDZ DOMAINS</u>	70
	5.5.1.2 <u>TPR AND RELATED DOMAINS</u>	71
	5.5.1.3 <u>PROLINE-SPECIFIC PEPTIDASES</u>	73
	5.5.1.4 <u>CLASS II MHC PROTEINS</u>	74
10	5.5.2 <u>ALTERATION OF CELLULAR PROTEIN FUNCTIONS</u>	74
	5.5.3 <u>ENZYMATIC BINDING SITES</u>	75
	5.5.4 <u>PROTEIN LIBRARIES/SUBSTRATES</u>	76
	5.5.5 <u>DIAGNOSTIC KITS</u>	79
15	5.6. <u>OTHER EMBODIMENTS/USES</u>	80
6. EXAMPLES	81	
6.1	<u>THE PDZ DOMAIN</u>	81
6.2	<u>PDZ DOMAIN REDESIGN</u>	82
6.2.1	<u>RE-DESIGN TO BIND NATURAL TARGET</u>	83
6.2.2	<u>RE-DESIGN TO BIND A KINESIN</u>	84
6.2.3	<u>SELECTION OF SEQUENCES TO BE TESTED</u>	
	<u>EXPERIMENTALLY</u>	86
6.3	<u>REDESIGNED PDZ DOMAIN ASSAYS</u>	87
6.3.1	<u>TWO-HYBRID ASSAYS</u>	89
6.3.2	<u>AFFINITY CHROMATOGRAPHY ASSAYS</u>	90
6.3.3	<u>MICRO-CALORIMETRIC ASSAY</u>	91
6.4.	<u>EXPRESSION IN MAMMALIAN CELLS</u>	91

30

35